IMPROVING THE EFFICIENCY OF HUMAN NEURAL STEM CELL DIFFERENTIATION BY TARGETING TRANSCRIPTION FACTORS TBR1 AND TBR2 WITH CRISPR-CAS9 GENOME EDITING

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Cell-based therapies are attractive for treating neurodegenerative diseases. Various neuronal cell lineages can be generated through stem cell differentiation to restore impaired functions. The process of differentiation depends on the presence or absence of specific transcription factors, which are cell lineage- dependent. For example, the transition between an intermediate progenitor to a postmitotic glutamatergic neuron has been correlated to the downregulation and subsequent upregulation of (T-Box, Brain) TBR2 and TBR1, respectively. This suggests that both TBR1 and TBR2 are important for the development of the glutamatergic neurons. Although different types of neuronal cells have been generated in vitro, these methods often lead to heterogeneous cell populations due to the generation of unintended/off-target cell types. Potential health risks have been associated with these off-target effects in human studies which impede the advancement of cell-based therapies. Thus, there is a need to more precisely control the lineage commitment of stem cells.

To address this challenge, we created knockout of specific transcription factors in a human embryonic H9-derived neural stem cell (hNSCs) line, to restrict their lineages and reduce the heterogeneity of the cell population. We used CRISPR-Cas9 to restrict the differentiation of NSC lineages by targeting TBR1 and TBR2. Fluorescence-activated cell sorting, used to quantify the neuronal population after differentiation, showed that NSCs lacking TBR1 or TBR2 reduced the percentage of glutamatergic neurons as compared to wild-type control under the same differentiation condition. Thus, this study indicate thatthe absence of specific transcription factors can restrict lineage commitment and thereby improvethe efficacy of cell-based therapy.